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Effects of pomegranate peel extract and vitamin E on oxidative stress and antioxidative capacity of hemodialysis patients: A randomized controlled clinical trial

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ABSTRACT

This study aimed to assess the effects of 8-week administration of pomegranate peel extract (PPE, ~180 mg ellagic acid) and vitamin E (Vit E, 400 IU) alone and in combination on oxidative stress (OS) and antioxidative capacity of hemodialysis (HD) patients. One hundred HD patients randomly divided in to 4 groups (control, PPE, Vit E, and PPE + Vit E). The changes of malondialdehyde, oxidized low-density lipoprotein, and myeloperoxidase in PPE + Vit E, PPE, and Vit E groups were significantly different compared to placebo group. The Changes of advanced oxidation protein products, superoxide dismutase, and oxygen radical absorbance capacity in PPE + Vit E group and changes of protein carbonyls in PPE and PPE + Vit E groups were significantly different compared to the other groups. As a result, consumption of PPE combined with Vit E was more effective than PPE or Vit E alone to ameliorate OS and enhance antioxidative capacity of HD patients.

1. Introduction

Oxidative stress (OS) occurs when the harmony between antioxidative capacity and production of oxidative agents disrupts in the body. In this situation, the accumulation of toxic products like reactive oxygen and nitrogen species, and other free radicals damage the cells and biomolecules like proteins, carbohydrates, lipids, and nucleic acids (Fallah, Sarmast, & Jafari, 2020; Jafari, 2016). Production of toxic substances regularly occurs in the metabolic pathways, but the antioxidative defense of the body confronts it. Antioxidative defense is disrupted in some situations like chronic inflammatory conditions, metabolic disorders, and progression of organ failure (Fallah, Sarmast, Fatehi, & Jafari, 2020).

Chronic kidney disease (CKD), defined as impaired renal function for more than 3 months, is one of the most serious life-threatening diseases worldwide. More than 500 million people throughout the world suffer from CKD (Levey & Coresh, 2012; McManus & Wynter-Minott, 2017). The prevalence of CKD is high in Asian countries. Results of a meta-analysis showed that the prevalence of CKD exceeds 15% in Iranian population and even higher in women (Bouya, Balouchi, Rafiemanesh, & Hesaraki, 2018). The prevalence of cardiovascular diseases (CVD) and its related mortality in CKD patients is remarkable (McManus & Wynter-Minott, 2017). Hemodialysis (HD) is a way to compensate for renal dysfunction in these patients. Although HD improves the longevity and quality of life, it does not resolve the problem of cardiovascular events in CKD subjects. Moreover, HD may increase the prevalence of CVD via the exacerbation of OS status and free radical production. For this reason, physicians are looking for treatments to enhance the antioxidative status in HD subjects (Jafari, 2016; Webster, Nagler, Morton, & Masson, 2017).

Recently, the tendency to use natural antioxidant compounds in HD patients has been increased. Pomegranate (*Punica granatum* L.) has received much attention due to its significant amount of natural antioxidants, such as polyphenols and flavonoids, and taught to have beneficial effects on human health especially, in patients with chronic diseases (Akhtar, Ismail, Fraternale, & Sestili, 2015; George et al., 2019;

Abbreviations: PPE, pomegranate peel extract; Vit E, vitamin E; OS, oxidative stress; HD, hemodialysis; Ox-LDL, oxidized low-density lipoprotein; AOPP, advanced oxidation protein products; MDA, malondialdehyde; PC, protein carbonyls; SOD, superoxide dismutase; MPO, myeloperoxidase; FRAP, ferric reducing antioxidant power; ORAC, oxygen radical absorbance capacity

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Table 1
Baseline characteristics of participants.

Variable	Intervention group				*P* value[e]
	Placebo[a]	PPE[b]	Vit E[c]	PPE + Vit E[d]	
Age (year)	56.4 ± 16.14	57.5 ± 17.02	52.0 ± 16.9	51.2 ± 12.9	0.285
Gender (female/male)	12/12	14/11	13/11	10/14	0.933
BMI (kg/m^2)	23.1 ± 3.90	23.6 ± 3.16	23.4 ± 3.17	24.6 ± 3.38	0.430
HD duration (month)	11.0 ± 6.74	9.33 ± 5.34	11.2 ± 7.44	10.8 ± 6.00	0.727
Systolic blood pressure (mmHg)	145.2 ± 17.9	147.0 ± 19.1	135.5 ± 21.0	142.7 ± 17.9	0.245
Diastolic blood pressure (mmHg)	71.1 ± 13.1	75.5 ± 15.4	83.5 ± 15.0	75.5 ± 12.6	0.121
Total cholesterol (mg/dl)	155.4 ± 33.1	165.4 ± 33.1	162.5 ± 25.4	166.5 ± 30.5	0.357
LDL (mg/dl)	98.0 ± 33.5	98.4 ± 27.8	97.4 ± 29.5	99.5 ± 45.1	0.564
Triglycerides (mg/dl)	145.3 ± 34.6	150.0 ± 32.5	140.5 ± 40.0	145.4 ± 32.0	0.255
CRP (mg/l)	18.8 ± 9.11	14.4 ± 7.60	17.2 ± 14.2	14.6 ± 6.48	0.329
Kt/v[f]	1.60 ± 0.10	1.50 ± 0.30	1.41 ± 0.71	1.61 ± 0.12	0.455

Note: Data are mean ± SD.
[a] Received 3 placebo pills.
[b] Received 2 pomegranate peel extract (PPE) tablets + 1 vitamin E (Vit E) placebo.
[c] Received 1 Vit E + 2 PPE placebo.
[d] Received 2 PPE tablets + 1 Vit E.
[e] Obtained from ANOVA.
[f] K shows dialyzer clearance of urea, t shows dialysis time, and V shows volume of distribution of urea.

Table 2
Dietary intakes of participants throughout the study.

Variable	Intervention group				*P* value[e]
	Placebo[a]	PPE[b]	Vit E[c]	PPE + Vit E[d]	
Energy (Kcal/day)	2125 ± 337	2205 ± 295	2157 ± 293	2175 ± 308	0.225
Carbohydrate (g/day)	325.5 ± 93.0	305.7 ± 87.6	319.7 ± 51.5	325.5 ± 71.6	0.846
Protein (g/day)	79.9 ± 16.5	85.2 ± 20.0	80.1 ± 19.5	79.2 ± 17.5	0.543
Fat (g/day)	84.2 ± 20	83.1 ± 19.7	79.8 ± 15.8	82.4 ± 16.9	0.127
PUFA (g/day)	16.3 ± 3.61	19.7 ± 7.42	15.2 ± 4.60	17.5 ± 3.72	0.625
Vitamin C (mg/day)	153.9 ± 10.2	150.1 ± 11.5	146.9 ± 10.6	147.4 ± 8.90	0.458
Vitamin E (mg/day)	5.34 ± 2.61	5.76 ± 3.44	6.01 ± 4.77	5.86 ± 3.50	0.795

Note: Data are mean ± SD, the average of 5 food recalls is represented for each food item.
[a] Received 3 placebo pills.
[b] Received 2 pomegranate peel extract (PPE) tablets + 1 vitamin E (Vit E) placebo.
[c] Received 1 Vit E + 2 PPE placebo.
[d] Received 2 PPE tablets + 1 Vit E.
[e] Obtained from ANOVA.

Liu et al., 2019). Pomegranate peels, the thin interior layer that surround the pomegranate seeds, are the richest source of flavonoids and tannins compared to the other parts of the fruit and characterized as a powerful natural antioxidant (Ismail, Sestili, & Akhtar, 2012; Wu et al., 2015).

Vitamin E (Vit E) composes of 8 components; 4 tocotrienols (TT) and 4 tocopherols (TP, α, β, γ, δ), which characterized as powerful natural antioxidants. The very weak O-H bonds in these components, especially in tocopherols, enable them as hydrogen providers and make them strong scavengers of free radicals and reactive oxygen species (ROS), which protect cell membrane against lipid peroxidation (Daud et al., 2013; Rusu, 2018). Studies have shown that the serum level of Vit E decreases in HD patients, hence Vit E administration can be useful for these patients (D'Arrigo, Baggetta, Tripepi, Galli, & Bolignano, 2017).

It has been demonstrated that HD causes excessive OS due the loss of antioxidants during dialysis procedure and accumulation of oxidative products (Liakopoulos, Roumeliotis, Gorny, Dounousi, & Mertens, 2017). Vit E is recommended to be used in the range of 400–800 IU/day for HD patients to prevent CVD (Rusu, 2018). Results of previous studies showed that administration of Vit E alone did not effectively improve OS status of HD patients, while combination of Vit E with other antioxidants ameliorated OS in these subjects (Ahmadi, Mazooji, Roozbeh, Mazloom, & Hasanzade, 2013; Diepeveen et al., 2005). In a study, consumption of pomegranate juice for a long time period ameliorated OS status in HD patients; moreover, it took at least 3 months for the beneficial antioxidative effects of pomegranate juice to become apparent (Shema-Didi et al., 2012). Considering the restriction of taking potassium in patients with renal failure, it seems that pomegranate juice, which contains high potassium levels, is not suitable for these subjects, while the use of pomegranate extracts especially pomegranate peel extract (PPE), which contains higher levels of antioxidants, can be a good alternative. Wu et al. (2015) found that administration of pomegranate extract enhanced antioxidative capacity of HD patients, while did not effectively improve OS status.

Combined supplementation of antioxidants may have synergistic, indifferent, or even antagonistic effects. Referring to the scientific literature, no human clinical trial was performed on the co-supplementation of Vit E and PPE. Therefore, this study aimed to assess the effects of PPE and Vit E alone and in combination on biomarkers of oxidative stress and antioxidative capacity of HD patients.

2. Materials and methods

2.1. Study protocol

This study was performed according to the guidelines of Helsinki declaration and approved by Ethical Committee of Research, Shahrekord University of Medical Sciences (IR.SKUMS.REC.1395.235).

Table 3
Comparison of baselines and after-intervention values in the study groups.

Variable	Placebo[a]			PPE[b]			Vit E[c]			PPE + Vit E[d]			
	Before	After	P^e	Before	After	P^e	Before	After	P^e	Before	After	P^e	P^f
Ox-LDL (ng/L)	4772.4 ± 1059.3	5201.1 ± 1087.6	0.020	5200.7 ± 1265.9	5009.0 ± 1280.4	0.112	4993.7 ± 1123.9	4794.2 ± 1212.1	0.124	5110.1 ± 1324.3	4781.1 ± 1211.4	0.020	0.625
MDA (μM/L)	169.8 ± 68.4	224.3 ± 71.2	0.001	213.51 ± 66.8	150.6 ± 68.6	< 0.001	196.2 ± 68.6	151.4 ± 64.5	0.015	202.0 ± 76.3	142.8 ± 65.8	0.001	0.168
AOPP (μM/L)	104.7 ± 22.1	121.3 ± 26.3	0.001	106.5 ± 25.9	99.4 ± 26.6	0.011	110.5 ± 20.1	112.6 ± 25.3	0.465	109.3 ± 35.5	87.2 ± 28.9	< 0.001	0.872
PC (nM/mg protein)	0.22 ± 0.14	0.32 ± 0.20	< 0.01	0.31 ± 0.24	0.28 ± 0.24	0.119	0.29 ± 0.23	0.32 ± 0.22	0.145	0.33 ± 0.27	0.20 ± 0.18	0.001	0.344
MPO (U/mg protein)	19.9 ± 9.19	25.5 ± 9.61	0.014	26.9 ± 9.67	25.7 ± 10.2	0.253	23.2 ± 7.54	22.0 ± 8.25	0.327	24.9 ± 8.89	19.8 ± 7.89	0.001	0.051
SOD (% inhibition)	24.2 ± 7.33	22.9 ± 6.68	0.218	20.5 ± 8.90	21.3 ± 10.5	0.396	23.0 ± 8.90	24.1 ± 9.09	0.423	21.3 ± 11.0	24.8 ± 11.6	0.001	0.498
FRAP (μM/L)	630.1 ± 177.6	612.2 ± 191.5	0.605	589.1 ± 146.7	594.8 ± 124.2	0.897	622.5 ± 154.5	625.5 ± 140.7	0.921	620.0 ± 152.3	699.4 ± 150.3	0.025	0.802
ORAC (μM/L)	912.1 ± 167.4	894.2 ± 170.1	0.340	899.8 ± 198.5	911.7 ± 201.6	0.242	904.9 ± 174.7	913.5 ± 187.1	0.569	910.9 ± 183.0	1001.5 ± 188.4	0.003	0.995

Note: Data are mean ± SD.
Abbreviations: Ox-LDL, oxidized low-density lipoprotein; MDA, malondialdehyde; AOPP, advanced oxidation protein products; PC, protein carbonyls; MPO, myeloperoxidase; SOD, superoxide dismutase; FRAP, ferric reducing antioxidant power; ORAC, oxygen radical absorbance capacity.
[a] Received 3 placebo pills.
[b] Received 2 pomegranate peel extract (PPE) tablets + 1 vitamin E (Vit E) placebo.
[c] Received 1 Vit E + 2 PPE placebo.
[d] Received 2 PPE tablets + 1 Vit E.
[e] Obtained from paired *t*-test. Shows within group change.
[f] Obtained from ANOVA. Shows between-group difference in baseline values.

This study was also recorded at www.irct.ir (registration ID: IRCT20180816040814N1). Participants were fully informed of the intervention protocol and they signed the written informed consent forms.

2.2. Randomization and blinding

In this randomized, double-blinded, placebo-controlled clinical trial, HD patients referred to HD centers in Chaharmahal-va-Bakhtiari province of Iran were enrolled. For group allocation, stratified block randomization procedure (Random Allocation Software) based on sex, age, and HD duration was used. The random sequence was generated by someone who was not involved in the intervention. The allocation was concealed till the end of the study. Each drug (pomegranate tablet or Vit E soft gel) and its placebo had identical shape, color, size, and packaging; and also coded with sequential numbers by someone who was not involved in the intervention. The interpretation of codes was concealed to the researchers till end of the analysis. All investigators, participants, and the laboratory technicians were blinded to the random assignments and content of the intervention.

2.3. Medicine preparation

Pomegranate tablets (ANAR®) containing 225 mg PPE (90 mg ellagic acid) were purchased from Amin Pharmaceutical Company, Iran. Vitamin E, 400 IU soft gels (dL-Alpha-Tocopheryl Acetate), were purchased from Zahravi Pharmaceutical Company, Iran. The pomegranate and vitamin E placebos were made by the same companies. Placebos were exactly the same as the original drug in terms of shape, color, size, and packaging.

2.4. Participants and study design

In this research, HD patients referred to HD centers in Chaharmahal-va-Bakhtiari province of Iran were enrolled. The inclusion criteria were as follows: patients undergoing HD for at least 3 months and 3 times a week, without infectious diseases, sever liver disorders, malignancies, or use drugs with definite antioxidative and antiinflammatory capacity, or definite interaction with vitamin E and pomegranate tablets. During the intervention, participants who did not tolerate the drugs, or altered their dietary patterns or drugs were excluded. The selected participants (based on inclusion criteria) were randomly divided in 4 equal parallel groups: group Pom + Vit E, which received 2 pomegranate tablets (450 mg PPE equal to 180 mg ellagic acid) + 1 Vit E soft gel (400 IU) daily; group Pom, which received 2 pomegranate tablets + 1 Vit E placebo soft gel daily; group Vit E, which received 1 Vit E soft gel + 2 pomegranate placebo tablets daily; and group placebo, which received 2 pomegranate placebo tablets + 1 Vit E placebo soft gel daily. After one-week run-in period to prepare participants, the intervention began and launched for 8 weeks. According to the information provided by the pharmaceutical company for consumption of PPE tablets (ANAR®), taking more than 2 tablets a day is not recommended for renal failure patients. Vit E is recommended to be used in the range of 400–800 IU/day for HD patients (Rusu, 2018). To determine the doses of PPE tablets and Vit E, the research team held a meeting with the medical treatment team of HD patients participating in this study. According to the treatment protocol of the patients, maximum daily intake of 2 PPE tablets and 400 mg of Vit E were allowed. A drug calendar was provided for participants and they were asked to tick the times they took their medicine. They were also asked to keep empty pockets of consumed medicines and deliver them on the visits. Patients were visited 3 times a week when they came for their HD schedule. The consumption of medicine in days of HD schedule was after the HD procedure.

To calculate the sample size, statistical formula for parallel clinical trials was used. Based on $\alpha = 0.05$, 90% power, and standardized effect size of malondialdehyde (MDA) as key variable (Shema-Didi et al.,

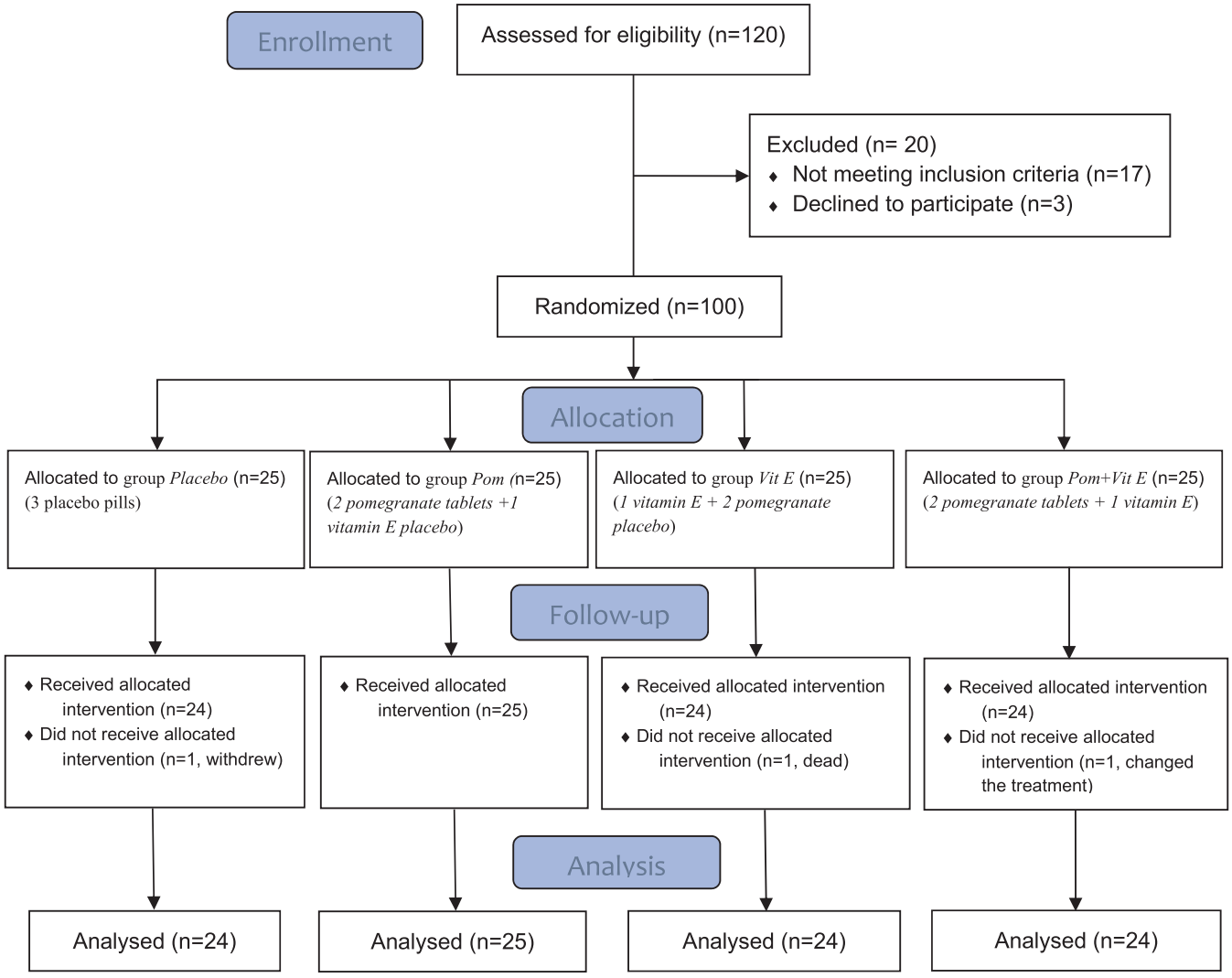

Fig. 1. CONSORT flowchart of study design.

Table 4
Comparison of changes in the study variables among the groups.

Variable	Intervention group/change from baseline value				*P* value[e]
	Placebo[a]	PPE[b]	Vit E[c]	PPE + Vit E[d]	
Ox-LDL (ng/L)	428.7 ± 840.3[w]	−190.9 ± 590.5[x]	−199.5 ± 612.5[x]	−329.2 ± 645.8[x]	< 0.001
MDA (μM/L)	54.5 ± 69.7[w]	−62.9 ± 77.6[x]	−45.7 ± 85.1[x]	−59.2 ± 71.8 [x]	< 0.001
AOPP (μM/L)	16.6 ± 22.1[w]	−7.10 ± 13.2[x]	2.11 ± 13.9[wx]	−22.2 ± 26.2[y]	< 0.001
PC (nM/mg protein)	0.10 ± 0.15[w]	−0.36 ± 0.11[y]	0.03 ± 0.09[wx]	−0.13 ± 0.16[y]	< 0.001
MPO (U/mg protein)	5.56 ± 10.2[w]	−1.16 ± 5.08[x]	−1.19 ± 5.85[x]	−5.09 ± 5.99[x]	< 0.001
SOD (% inhibition)	−1.21 ± 4.69[w]	0.78 ± 4.61[w]	1.07 ± 6.45[w]	3.51 ± 4.60[x]	0.015
FRAP (μM/L)	−17.9 ± 167.2[w]	5.73 ± 190.2[w]	4.02 ± 195.6[w]	79.4 ± 162.0[w]	0.116
ORAC (μM/L)	−17.9 ± 90.1[w]	11.9 ± 50.6[w]	8.54 ± 72.4[w]	90.5 ± 131.9[x]	0.001

Note: Data are mean ± SD.
Abbreviations: Ox-LDL, oxidized low-density lipoprotein; MDA, malondialdehyde; AOPP, advanced oxidation protein products; PC, protein carbonyls; MPO, myeloperoxidase; SOD, superoxide dismutase; FRAP, ferric reducing antioxidant power; ORAC, oxygen radical absorbance capacity.
[a] Received 3 placebo pills.
[b] Received 2 pomegranate peel extract (PPE) tablets + 1 vitamin E (Vit E) placebo.
[c] Received 1 Vit E + 2 PPE placebo.
[d] Received 2 PPE tablets + 1 Vit E.
[e] obtained from MANCOVA.
[w,x,y] The values of each marker with different superscript letters are significantly different among the groups ($P < 0.05$).

2012), 17 subjects on each group were needed. To avoid losing samples during the intervention period, 25 subjects were considered for each group.

2.5. Dietary assessment

Dietary intakes of the patients were monitored to make sure that they did not change their usual diet. Dietary recalls were taken at the beginning, weeks 2, 4, 6, and at the end of the intervention period (including a weekend) using a 24-h recall questionnaire. To calculate energy and nutrients, Nutritionist 4 software (based on United States Department of Agriculture (USDA) food composition table modified for Iranian foods) was used. The average of 5-day dietary recalls was expressed as dietary intake.

2.6. Anthropometric measurements

Weight of participants, with light clothes and without shoes, was measured before HD using a digital scale (Seca, Hamburg, Germany). Height was measured with a stadiometer to the nearest of 0.1 cm. BMI was calculated as weight in kg/(height in meter)^2. Weight and height were measured after overnight fasting status (Jafari et al., 2018).

2.7. Laboratory measurements

Blood samples were taken at the beginning and end of the intervention prior to dialysis. The sera samples were isolated and kept at −80 °C before analyses. Lipid peroxidation was assessed by determination of oxidized low-density lipoprotein (Ox-LDL) and malondialdehyde (MDA). A commercial ELISA kit (Eastbiopharm Company, USA) was used to determine the levels of Ox-LDA, while method of Morel, DiCorleto, and Chisolm (1984) was used to determine MDA levels. Protein oxidation was assessed by determining the levels of advanced oxidation protein products (AOPP) according to the method of Witko-Sarsat et al. (1996) and protein carbonyls (PC) based on the method of Levine et al. (1990). Superoxide dismutase (SOD) activity was determined by the method of Nakayama, Akiyama, Inagaki, Gotoh, and Oguchi (2001). Serum level of myeloperoxidase (MPO), a member of peroxidases, was assessed by a commercial ELISA kit (Eastbiopharm Company, USA). Total antioxidant capacity was assessed by determination of Ferric reducing antioxidant power (FRAP) according to the method of Benzie and Strain (1996) and oxygen radical absorbance capacity (ORAC) based on the method of Prior et al. (2003).

2.8. Statistical analyses

Quantitative data were presented as mean ± SD, and qualitative data as number and percentage. Normality of studied variables was assessed by Kolmogorove-Smirnov test and Q–Q plot. One-way analysis of variance (ANOVA) was used to detect between group differences in general characteristics, dietary intakes, and OS biomarkers at baseline. To determine the effects of the intervention on OS biomarkers and antioxidative defense, we used multivariate analysis of covariance (MANCOVA). Changes from baseline values across the four groups were compared. To adjust the probable effects of confounding factors on the results, we considered baseline values, age, gender, baseline BMI, and HD duration as covariates in the analyses. *P*-values < 0.050 were considered statistically significant. Statistical analyses were performed using the Statistical Package for Social Science version 20 (SPSS Inc., Chicago, IL, USA).

3. Results

One hundred and twenty-five medical records of HD patients who were interested to participate in the study were evaluated and 100 patients were selected according to the inclusion criteria. They were randomly enrolled in 4 groups. Baseline characteristics of participants are shown in Table 1. The groups were equal in number of participants (25 persons in each group) and there were no statistically significant differences between them in age, gender, HD duration, and adequacy (expressed by Kt/V index). Participants were 55 males and 45 females with an average age of 56 years and mean HD duration of ~10.6 months (Table 1). Mean total energy and nutrient intakes did not show significant differences among the groups (Table 2). No significant difference was found in baseline values of Ox-LDL, MDA, AOPP, PC, MPO, SOD, FRAP, and ORAC among the groups (Table 3, *P* value obtained from ANOVA). Three participants were excluded from the study (one died in the second week of intervention, one withdrew, and one excluded due to change in treatment) and 97 participants completed the intervention period. Fig. 1 shows the intervention process.

Table 3 shows the baseline variables and their values after an 8-week intervention. Results of paired t-tests represented that serum level of Ox-LDL significantly decreased in PEE + Vit E group, while significantly increased in placebo group compared to the baseline values ($P < 0.05$). In PPE and Vit E groups, serum level of Ox-LDL did not significantly change ($P > 0.05$) compared to the baseline values. Serum level of MDA significantly decreased in PPE + Vit E, Vit E, and PPE groups, while significantly increased in the placebo group compared to the baseline values ($P < 0.05$). Serum levels of AOPP and MPO significantly decreased in PPE + Vit E and PPE groups, while significantly increased in placebo group compared to the baseline values ($P < 0.05$). In Vit E group, serum level of AOPP and MPO did not significantly change ($P > 0.05$) compared to the baseline value. Serum level of PC significantly decreased in PPE + Vit E group, while significantly increased in placebo group compared to the baseline values ($P < 0.05$). In PPE and Vit E groups, Serum level of PC did not significantly change ($P > 0.05$) compared to the baseline value. The SOD activity and FRAP and ORAC levels significantly increased ($P < 0.05$) in PPE + Vit E group, while did not significantly change ($P > 0.05$) in the other groups compared to the baseline values (Table 3).

In general, results represented that after adjustments for the baseline values, age, gender, and HD duration, our 8-week interventions significantly reduced OS and improved antioxidative defense in HD patients (Wilks Lambda $P < 0.001$). Table 4 shows the changes from baselines values of study variables. The *P* values obtained from MANCOVA for each variable revealed significant differences among the groups except for FRAP (Table 4). The pairwise comparisons represented that changes in Ox-LDL, MDA, and MPO values of PPE + Vit E, PPE, and Vit E groups showed significant differences compared to the change of the placebo group. The Changes of AOPP and SOD values in PPE + Vit E group and changes of PC value in PPE and PPE + Vit E groups showed significant differences compared to the other groups. The changes of FRAP value did not show significant difference among the groups, while the change of ORAC value in PPE + Vit E group was significantly different from the changes in the other groups (Table 4).

4. Discussion

This study showed that daily consumption of 450 mg PPE combined with 400 IU Vit E for 8 weeks was more effective than consumption of PPE or Vit E alone to ameliorate OS in HD patients. The combination therapy reduced the values of lipid and protein peroxidation indices as well as the value of MPO and increased the value of SOD. The decrease in the value of Ox-LDL was more pronounced in the PPE + Vit E group compared to the PPE and Vit E groups. The levels of MDA significantly increased in the placebo group while decreased in the other groups.

The decrease in the value of MPO was also more pronounced in patients who received PPE + Vit E compared to those who received Vit E alone. The increase in the value of SOD was more pronounced in the PPE + Vit E group compared to the other groups. The ORAC and FRAP are considered as the biomarkers of total antioxidant capacity (Cao & Prior, 1998). The values of ORAC showed significant changes between

the 4 groups; moreover, it was significantly increased in PPE + Vit E group. FRAP value notably increased in patients who received PPE + Vit E compared to the baseline value. These effects might be due to the additive or synergistic antioxidative activities of PPE and Vit E.

Patients undergoing HD therapy are exposed to developing OS condition. The chronic and debilitating nature of the procedure and its side effects in addition to high inflammation, malnutrition, and multi-drug consumption impair the antioxidant defense and increase the accumulation of toxins, ROS, and other pro-oxidant components in the body. Such conditions lead the HD patients to develop CVD (Sakaguchi et al., 2014). Enhancement of antioxidant defense and reduction of OS and inflammation in these patients can improve their health status.

Results of previous human clinical trials on the antioxidative effects of Vit E supplementation in HD patients are contradictory. Some studies represented that administration of Vit E reduced ROS synthesis, lipid peroxidation, and platelet aggregation (Mune et al., 2018; Sohrabi, Eftekhari, Akbarzadeh, Eskandari, & Sagheb, 2017) while some others failed to show the antioxidative effects of Vit E in HD subjects (Diepeveen et al., 2005; Kamgar, Zaldivar, Vaziri, & Pahl, 2009; Lu, Erhard, Salomon, & Weiss, 2007). The function of Vit E in the body is influenced by several factors like the amount of Vit E consumed, the duration of consumption, and the vitamin metabolism in the body (Daud et al., 2013). It has been recommended to use 400–800 IU/day of Vit E for HD patients (D'Arrigo et al., 2017). There is remarkable heterogeneity among the studies reporting Vit E doses. Some of them used International unit/day (IU/day) while the others used mg/day. In general, the administered Vit E doses varied from 300 to 800 IU/day or 200 to 800 mg/day and the intervention durations launched from 1 week to 1 year. In addition, considering the malnutrition and impaired normal physiology of digestion, absorption, and metabolism in HD patients, the bioavailability of Vit E in these patients is undesirable. For these reasons, an adjunctive antioxidative treatment seems to be necessary.

Shema-Didi et al. (2012) showed that daily consumption of 100 ml pomegranate juice (containing 0.7 mmol polyphenols) for one year, ameliorated OS indices i.e. MDA, AOPP, and MPO in HD patients. In another study, pomegranate juice attenuated the exacerbation of OS status induced by intra-venous iron administration in these patients (Shema-Didi et al., 2013). In a cross-over study, Rivara et al. (2015) evaluated the effects of pomegranate juice (100 ml/day for 4 weeks) and pomegranate extract tablets (1250 mg/day for 4 weeks) in 24 HD patients. The washout period between the 2 interventions was 4 weeks. They reported that neither pomegranate juice nor pomegranate extract significantly changed the OS markers as well as inflammatory markers in these subjects. Wu et al. (2015) reported that 6-month pomegranate extract supplementation (1000 mg/day capsule of purified pomegranate polyphenol extract, equivalent to 600–755 mg gallic acid) had no effects on OS markers, blood pressure, and cardiovascular risk factors in HD patients, but enhanced antioxidative capacity of the subjects. The differences in type, amount, and the duration of the intervention as well as the heterogeneity of pomegranate bioavailability in patients may be the reasons of the contradiction.

Overall, results of previous clinical trials revealed that administration of Vit E (400–800 IU/day) for a short or long time period (8 weeks to 6 months) did not effectively ameliorate OS status of HD patients (Ahmadi et al., 2013; Diepeveen et al., 2005; Kamgar et al., 2009; Lu et al., 2007). Considering pomegranate products, Shema-Didi et al. (2012) found that it took at least 3 months for the antioxidative effects of pomegranate juice consumption to appear in HD patients. In contrast, Wu et al. (2015) found that 6-month consumption of pomegranate extract did not effectively ameliorate OS status of HD patients. Results of our study showed that administration of Vit E and PPE alone did not effectively improve biomarkers of OS and antioxidative capacity of HD patients after an 8-week intervention, while combination therapy considerably reduced OS and enhanced antioxidative capacity of the subjects. This can be a valuable result and considered as the novelty of this study because combination therapy of HD patients with Vit E and PPE efficiently improved the mentioned biomarkers after a short period of time, as a result of additive or synergistic effects of these compounds. This study had limitations as follows: our patient's conditions and their plan of treatment did not let us to extend the intervention duration more than 8 weeks. We also could not administer more than 400 IU/day of vitamin E due to the patients' treatment protocol.

5. Conclusions

In general, our 8-week interventions (pomegranate tablets, Vit E soft gel, and their combination therapy) were well tolerated in HD patients. We concluded that combination therapy (PPE + Vit E) is more effective than single therapy in the amelioration of OS condition and improve the health status of HD patients. More studies with longer intervention period of time and different doses of drugs are needed to manifest the beneficial antioxidant therapy of the mentioned reagents. We also recommend studies to investigate the potential benefits of the same interventions on the other type of patients with renal failure like patients with chronic kidney disease or on earlier stages of renal failure.

Ethical statement

We declare that this study has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) in accordance with experiments involving humans. This research also approved by Ethical Committee of Research, Shahrekord University of Medical Sciences (IR.SKUMS.REC.1395.235) and recorded at www.irct.ir (registration ID: IRCT20180816040814N1). Participants were fully informed of the intervention protocol and they signed the written informed consent forms.

CRediT authorship contribution statement

Tina Jafari: Conceptualization, Formal analysis, Investigation, Writing - original draft, Supervision. **Aziz A. Fallah:** Conceptualization, Methodology, Writing - review & editing, Project administration. **Mohsen Bahrami:** Investigation. **Zahra Lorigooini:** Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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